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Research article

The increase of current atmospheric CO_2 and temperature can benefit leaf gas exchanges, carbohydrate content and growth in C4 grass invaders of the Cerrado biome

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ABSTRACT

Leaf gas exchanges, carbohydrate metabolism and growth of three Brazilian Cerrado invasive African grasses were evaluated after growing for 75 days under doubled CO_2 concentration and temperature elevated by 3 °C. Results showed that although the species presented photosynthetic C4 metabolism, they all had some kind of positive response to increased CO_2. *Urochloa brizantha* and *Megathyrsus maximus* showed increased height for all induced environmental conditions. *Urochloa decumbens* showed only improvement in water use efficiency (WUE), while *U. brizantha* showed increased CO_2 assimilation and *M. maximus* presented higher biomass accumulation under doubled CO_2 concentration. The most significant improvement of increased CO_2 in all three species appears to be the increase in WUE. This improvement probably explains the positive increase of photosynthesis and biomass accumulation presented by *U. brizantha* and *M. maximus*, respectively. The increase in temperature affected leaf carbohydrate content of *M. maximus* by reducing sucrose, glucose and fructose content. These reductions were not related to thermal stress since photosynthesis and growth were not harmed. Cellulose content was not affected in any of the three species, just the lignin content in *U. decumbens* and *M. maximus*. All treatments promoted lignin content reduction in *U. brizantha*, suggesting a delay in leaf maturation of this species. Together, the results indicate that climate change may differentially promote changes in leaf gas exchanges, carbohydrate content and growth in C4 plant species studied and all of them could benefit in some way from these changes, constituting a threat to the native Cerrado biodiversity.

1. Introduction

The Brazilian Cerrado is the second largest phytogeographical domain in area, surpassed only by the Amazon rainforest, and one of the richest savanna biomes of the world, with high levels of endemism, being considered an extremely important area for conservation (Myers et al., 2000). However, much of this biodiversity is being lost because of biological invasions. Species of African grasses used as forage are spreading rapidly in the Cerrado fragments, probably displacing native species and therefore constituting a threat to the native biodiversity (Pivello et al., 1999). This problem is likely to be aggravated by other factors, such as climate change with simultaneous increases in atmospheric carbon dioxide (CO_2) concentration and in average global temperature (Baruch and Jackson, 2005).

Temperature and atmospheric CO_2 are important environmental drivers affecting plant growth, development and function, what can potentially change the composition and functioning of communities and ecosystems (Díaz et al., 2004), and both have changed in the recent past (Eller et al., 2012), due to the burning of fossil fuels and change in land use, among other causes (IPCC, 2013). In November 2017, mean atmospheric CO_2 concentration was 405.14 μmol mol^{-1} in Mauna Loa, Hawaii (USA) (ESRL, 2017) and 936 μmol mol^{-1} is expected at the end of the 21st century, considering the most pessimistic emission scenario (IPCC, 2013). At the same time, the last three decades has been successively warmer at the Earth's surface than any preceding decade since 1850 and it is predicted that global temperatures will continue to rise, reaching increases between 1.1 and 4.8 °C by 2100 (IPCC, 2013).

Therefore, concerns about how plants and natural ecosystems will respond to such changes are relevant to the current scientific agenda, since climate change is already responsible for changes in species distribution (Lenoir et al., 2008). Many groups have focused on studying the effects of increasing CO_2 and temperature, together or separately, in

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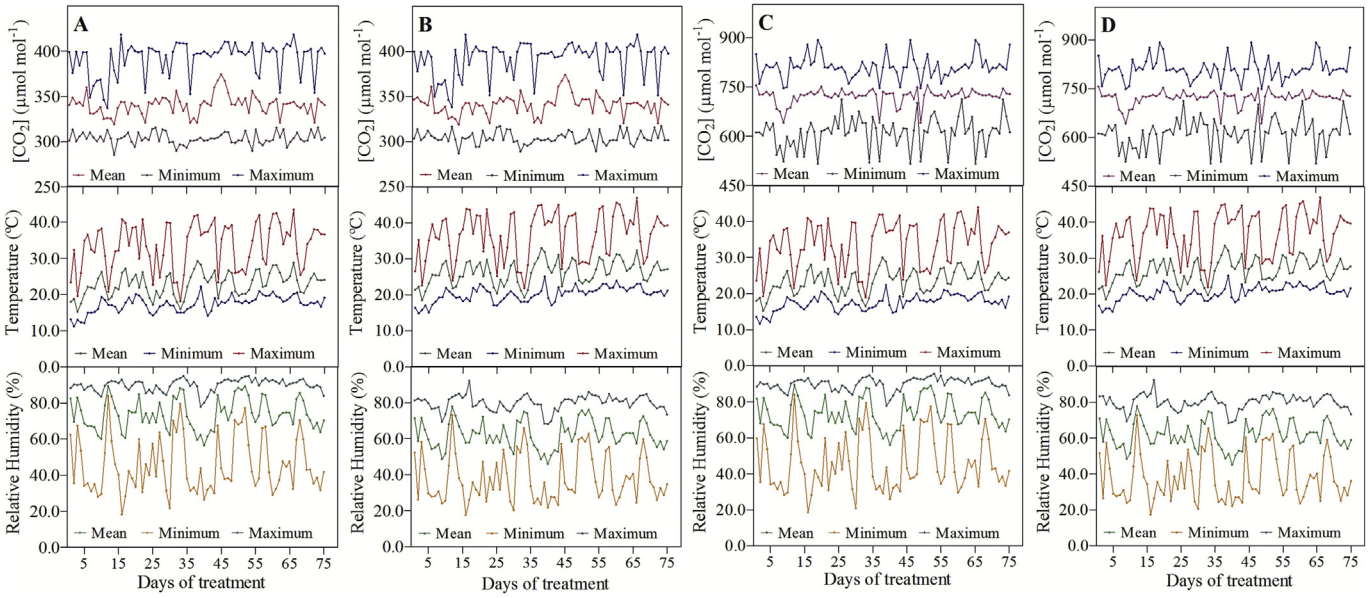

Fig. 1. Environmental conditions (CO_2 concentration, temperature and relative humidity) during 75 days of exposure to different environmental conditions. Each environmental condition is identified as a set of figures: A – Ctrl (Control: current CO_2 concentration and room temperature); B – ET (Elevated temperature: current CO_2 concentration and room temperature + 3 °C); C – EC (Elevated CO_2: doubled CO_2 concentration and room temperature) and D – ECT (Elevated CO_2 + temperature: doubled CO_2 concentration and room temperature + 3 °C), respectively.

plants and ecosystems, especially regarding photosynthetic and growth performance (Eller et al., 2012; Grombone-Guaratini et al., 2013; Souza et al., 2013; Kimbal, 2016; Runion et al., 2016).

Climate change may affect the productivity of the biota not only in relation to growth and resource allocation, but also changing the chemical composition of plant tissues (IPCC, 2013). Most source-sink hypotheses assume that high CO_2 concentration promotes a relative increase in carbon availability, which is accumulated on total non-structural carbohydrates and carbon-based secondary metabolites, provided that carbon values exceed growth requirements (Peñuelas and Estiarte, 1998). Carbon allocation for growth and partition should, by competition for internal resource of limited availability, reduce carbon allocation to secondary metabolism (Ibrahim and Jaafar, 2012). Among the works that investigated the influence of climate change on carbon metabolism in plants, some of them found that higher CO_2 concentration increased production of non-structural carbohydrates as starch (Ibrahim and Jaafar, 2012), and fructans (Oliveira et al., 2010). For sucrose, both increase and decrease of its content have been reported (Ibrahim and Jaafar, 2012; Grombone-Guaratini et al., 2013). For structural carbohydrates, Schädel et al. (2010) found that the increased CO_2 concentration had no significant effect on total hemicellulose concentrations in leaves and woody tissue in 14 of 16 species. Körner et al. (2005) have analyzed the litter composition of a temperate deciduous forest exposed to 530 ppm of CO_2 during 4 years in a FACE system (Free Air CO_2 Enrichment). They observed an increase of 21% in non-structural carbohydrate content and a decrease of 11% on lignin content. As for the increase of temperature, some studies report less accumulation of non-structural carbohydrates such as fructose and glucose in tomato leaves (Jie et al., 2012) and in grasses (Naudts et al., 2014).

Since the effects of CO_2 and temperature on plant metabolism may counteract each other, the combined effects can be different from any factor separately (Morison and Lawlor, 1999). As these climatic factors will change simultaneously, to understand how plants will respond and adapt to a new environment is an essential first step to understand the full impact that multiple climate change factors will have on terrestrial ecosystems (Díaz et al., 2004; Leakey et al., 2009; Eller et al., 2012). Thus, the present study aimed to investigate the effects of increases in CO_2 concentration and temperature predicted for 2100 by the Intergovernmental Panel on Climate Change (IPCC, 2013). The hypothesis is that these environmental changes can differentially affect gas exchanges, leaf carbohydrate content and growth of three of the most invasive species in the Brazilian Cerrado: *Urochloa brizantha*, *Urochloa decumbens* and *Megathyrsus maximus*, cultivated in a CO_2 enriched atmosphere and temperature elevated in 3 °C above room temperature.

2. Materials and methods

2.1. Plant material and environmental conditions

Plants of three grass species, *Urochloa brizantha* (Hochst. ex A. Rich.) RD Webster cv Marandu, *U. decumbens* (Stapf) RD Webster cv Basilisk and *Megathyrsus maximus* (Jacq.) BK Simon and S.W.L. Jacobs cv Tanzania were used in this study. Seeds of the all species were germinated in chambers at 30 °C, 12 h light and 12 h dark photoperiod. Three days after germination, seedlings were placed in 1.7 L plastic pots containing a mixture of sand and vermiculite (2:1) as substrate and grown for 75 days between October and December 2015 in open-top chambers (1.53 m^3 each), inside a glasshouse, designed according to Oliveira et al. (2010). Plants were irrigated with Hoagland and Arnon (1950) nutrient solution every three days and with distillated water on the other days. Four environmental treatments based on IPCC (2013) predictions for 2100 were imposed to the growing plants: (1) Control (Ctrl) – current CO_2 concentration (345.43 ± 38.59 µmol mol^{-1}) and room temperature (minimum 11.2 °C; mean: 22.9 °C and maximum: 43.5 °C); (2) Elevated temperature (ET) – current CO_2 concentration (345.55 ± 38.47 µmol mol^{-1}) and 3 °C above room temperature; (3) Elevated CO_2 (EC) – doubled CO_2 concentration (714.63 ± 89.40 µmol mol^{-1}) and room temperature; and (4) Elevated CO_2 + temperature (ECT) – double CO_2 concentration (724.56 ± 88.85 µmol mol^{-1}) and 3 °C above room temperature. The experiments were conducted under natural photoperiod and relative air humidity (RH). The environmental conditions (CO_2 concentration, temperature, RH and light intensity) were monitored throughout the experimental period (Fig. 1).

2.2. Plant growth evaluations

Four plants of each species and in each environmental treatment were measured at the 75th day of growth for allometric growth analysis. These consisted of the number of tillers, total plant height, number of leaves and culm diameter. Total plant height was measured using a measuring tape and culm diameter was measured at the plants base, just above the ground, using a digital caliper (Mitutoyo – Digimatic Caliper, 0.01–150 mm/0.0005″–6″, 500-144B). Four plants of each species and in each environmental treatment were harvested at the 75th day of growth. Biomass analysis consisted of accumulation of dry matter (total dry mass, including roots), above-ground biomass (culms and leaves) and shoot/root ratio, and leaf growth consisted of leaf dry mass (LDM) and leaf area (LA). To obtain LA, all plant leaves were newly collected and scanned, and the area calculated by AxioVision 4.9.1 (Zeiss) software. For biomass analysis, leaves, stems and roots were oven-dried at 65 °C in a forced air circulation drying kiln until they reach constant weight and weighted on an analytical scale (Shimadzu AY220).

2.3. Gas exchange measurements

Net carbon assimilation (*A*), stomatal conductance (g_s) and leaf transpiration (*E*) were measured using a portable photosynthesis system (LI-COR LI-6400XT) from 9 a.m. to 12 p.m. All measurements were performed under a photosynthetic photon flux density of 1600 μmol m^{-2} s^{-1}, determined as light saturation condition for the three species (Ziska et al., 1999; Dias-Filho, 2002; Gómez et al., 2013), and taken from six plants of each species and in each environmental condition from the middle portion of the first fully expanded leaf. CO_2 concentrations were maintained as constant during the measurements through the use of LI-6400XT CO_2 ampoules. For current CO_2 concentration, 360 μmol mol^{-1} concentration was used and 720 μmol mol^{-1} was used for elevated CO_2 and the measurements were taken using room temperature. Instantaneous water use efficiency was defined at leaf level as the ratio of photosynthetic carbon gain (*A*) to transpiration water loss (*E*), *A*/*E* (Donovan and Ehleringer, 1994).

2.4. Non-structural carbohydrates content

Leaf samples were freeze-dried and ground in a ball mill. Three hundred mg of each sample were extracted five times in 3 mL of 80% ethanol at 80 °C for 15 min, followed by centrifugation at 13,000 g for 10 min. The pooled supernatants were vacuum-dried and re-suspended in deionized water. Aliquots of ethanol extracts were purified through anion exchange columns (Dowex) and soluble sugars were analyzed by anion exchange chromatography coupled with pulsed amperometric detection (HPAEC/PAD) using an ICS 3000 Dionex system with a CarboPac PA-1 column (2 × 250 mm) using isocratic 12 mM NaOH. Different soluble sugars were identified by comparison with authentic standards (Sigma). Starch was quantified in the residue resulting from the extraction of soluble sugars, according to the method described by Amaral et al. (2007). Starch was hydrolyzed by sequential digestion with thermostable α-amylase from *Bacillus licheniformis* and amyloglucosidase (AMG) from *Aspergillus niger* (Megazyme). The glucose released was measured by mixing samples with glucose oxidase, peroxidase and 4-aminoantipyrine and phenol reagents and incubating at 30 °C for 15 min (Glucose PAP Liquiform, CENTERLAB). This reaction was read at 490 nm, using glucose as standard. Glucose released was adjusted (−10%) to the mass of linked glucose that is present in starch.

2.5. Cellulose and lignin content

Samples of 100 mg of freeze-dried ground leaves were used for cellulose and lignin dosage. Lignin content was determined by Klason's method (Hatfield et al., 1994 modified). Powdered material was re-suspended in 100 μL of 72% sulfuric acid and incubated in a water bath at 30 °C for 45 min. After incubation, the acid was diluted to 4% with distilled water and the samples were autoclaved for 1 h at 121 °C. The hydrolysate was centrifuged at 2000 g and the supernatant was discarded. The residue was washed three times in hot distilled water (~40 °C), dried at 40 °C and weighed on analytical balance (Shimadzu AY220). Dry mass corresponds to insoluble Klason's lignin. For cellulose content, samples were extracted with NaOH 8 M containing NaBH4 (0.4 mg mL^{-1}) for 1 h at room temperature. After extraction, samples were centrifuged at 2000 g for 20 min and supernatant discarded. This process was repeated overnight, with new centrifugation and discarding supernatant steps. Residue was re-suspended in water and neutralized with glacial acetic acid to pH between 6 and 8. After neutralization, samples were washed three times in distilled water with subsequent centrifugation at 2000*g* for 20 min, discarding supernatant and drying the residue at 40 °C. Insoluble material was digested in acetic-nitric acid at 100 °C for 1 h. The cellulose was washed three times in water, dried at 40 °C and weighed on analytical balance (Updegraff, 1969).

2.6. Statistical analysis

Data were analyzed by General Linear Model (GLM) and means were compared by contrast test at 5% probability using R 3.0.0 (free software). For all data, the environmental conditions were used as explanatory variables. The response variables used the number of tillers, total plant height, number of leaves and culm diameter for growth parameters; total dry mass and root/shoot ratio for biomass analysis; LDM and LA for leaf analysis; *A*, gs, *E* and WUE for gas exchange analysis and sucrose, glucose, fructose, starch, lignin and cellulose content for biochemical analysis. In addition to GLM analysis, all variables were analyzed by principal component analysis (PCA) using R 3.0.0 for all species studied and for each species separately. First PCA (all species and variables) proceeded on a data matrix of 48 lines (samples) and 18 variables comprising four growth parameters, two biomass parameters, two leaf parameters, four gas exchange parameters and six biochemical parameters to determine correlations between variables, treatments and species. The others PCA (each species separately) proceeded on a data matrix of 16 lines (samples) and 15 variables the following: one growth parameter (the one that showed statistical significance), two biomass parameters, two leaf parameters, four gas exchange parameters and six biochemical parameters to determine correlations between variables and treatments. Data was standardized by subtracting the means of values for each variable, from each variable value and dividing the result by the standard deviation of the values for each variable (Hossain et al., 2011). The purpose of standardization was to make sure that each parameter carried an equal weight in the principal component calculation.

3. Results

3.1. Plant growth evaluation

Urochloa decumbens showed no changes in any of the allometric parameters evaluated while growing under environmental conditions of increased CO_2 and/or temperature. Total plant height was the only parameter affected by different environmental conditions for *U. brizantha* and *M. maximus*. All environmental conditions resulted in increased total plant height for both species. For *U. brizantha*, total height was 18.4% and 17.2% higher for plants grown under elevated temperature (ET) and elevated CO_2 and temperature (ECT), respectively, than plants grown under control treatment (Ctrl). Also, total height was even higher for plants grown under elevated CO_2 (EC), showing an increase of 43.2%. *Megathyrsus maximus* was the species that benefited most from the environmental treatments effects in relation to the total height of plants. For all environmental treatments, increases in plant height showed the same magnitude, being more than 51% higher than

Table 1
Allometric growth parameters of three invasive grasses species grown under elevated CO_2 concentration and/or elevated temperature treatments.

	Number of tillers	Height (cm)	Number of leaves	Culm diameter (mm)
Urochloa brizantha				
Ctrl	2.50 ± 0.29	62.90 ± 1.58 **c**	10.75 ± 1.11	4.50 ± 0.00
ET	2.25 ± 0.25	74.50 ± 1.51 **b**	11.25 ± 0.85	4.00 ± 0.20
EC	2.25 ± 0.25	90.05 ± 2.32 **a**	12.25 ± 0.48	4.63 ± 0.25
ECT	2.75 ± 0.25	73.75 ± 4.89 **b**	13.25 ± 0.63	3.50 ± 0.20
Urochloa decumbens				
Ctrl	2.25 ± 0.25	70.85 ± 2.85	9.75 ± 0.63	2.13 ± 0.13
ET	4.00 ± 0.41	66.88 ± 1.48	14.75 ± 0.85	2.00 ± 0.00
EC	3.25 ± 0.75	72.30 ± 2.55	12.75 ± 1.55	2.00 ± 0.00
ECT	3.25 ± 0.25	73.75 ± 2.50	13.50 ± 1.50	2.13 ± 0.13
Megathyrsus maximus				
Ctrl	2.00 ± 0.41	29.13 ± 2.71 **b**	5.25 ± 0.25	7.00 ± 0.20
ET	3.00 ± 0.00	46.48 ± 1.29 **a**	7.00 ± 1.22	6.50 ± 0.89
EC	2.00 ± 0.00	44.15 ± 3.49 **a**	7.00 ± 0.41	7.13 ± 0.90
ECT	2.00 ± 0.41	45.05 ± 2.51 **a**	4.75 ± 0.25	5.75 ± 0.66

Ctrl: Control; ET: Elevated temperature; EC: Elevated CO_2; ECT: Elevated CO_2 + temperature. Results represent mean ± SEM (n = 4). Absence of letters indicate no statistical differences; different letters indicate statistical differences ($P < 0.05$) between environmental treatments.

plants of control treatment (Table 1).

Urochloa brizantha and *U. decumbens* did not show significant changes in total dry mass when subjected to increased CO_2 and/or temperature. Contrastingly, for *M. maximus*, elevated CO_2 led to increased total dry mass. For *M. maximus* grown under elevated CO_2 dry mass was 27.0% greater than those under control, 20.5% greater than those under elevated temperature and 56.7% greater than those under elevated CO_2 + temperature treatments (Fig. 2A). Despite the absence of significant differences in total dry mass, *U. brizantha* showed differences in above-ground biomass. The treatments with increased CO_2 (EC and ECT) resulted in larger above-ground biomass than those presented in control and ET treatments. For *U. decumbens* and *M. maximus*, the values followed the same trend presented by total dry mass (Fig. 2B). Regarding root/shoot ratio, for all studied species and all environmental treatment plants showed equally reduced root/shoot ratio values. *U. brizantha*, *U. decumbens* and *M. maximus* grown under elevated temperature showed root/shoot ratio values reduced in 36.2, 44.5 and 53.9%; under elevated CO_2 showed values reduced in 49.5, 23.7 and 39.9% and elevated CO_2 + temperature showed values reduced in 52.7, 41.2 and 55.6% related to those grown under control condition, respectively (Fig. 2C). For leaf growth parameters (Fig. 3), although they had no significant difference in total dry mass, *U. brizantha* plants showed greater leaf dry mass in treatments with increased CO_2 (EC and ECT). Plants subjected to elevated CO_2 and elevated CO_2 + temperature had leaf dry mass 54.5% greater than those under control treatment and 21.4% greater than those subjected to elevated temperature. *U. decumbens* and *M. maximus* did not show differences among treatments for leaf dry mass (Fig. 3A) and none of the induced environmental conditions significantly affected the leaf area of the three species (Fig. 3B).

3.2. Gas exchange evaluation

Urochloa brizantha was the only species to show net carbon assimilation (*A*) enhanced by elevated CO_2 treatments (EC and ECT). Elevated CO_2 + temperature were responsible for the best photosynthetic performance of this species. Elevated temperature induced higher stomatal conductance (g_s) in leaves of all species and elevated CO_2 + temperature also increased g_s in *U. brizantha* leaves. Transpiration rate (*E*) was also increased under elevated temperature in *U. decumbens* and *M. maximus* leaves, while elevated CO_2 + temperature promoted the same response in *U. brizantha*. Despite no differences observed in g_s and *E* parameters, water use efficiency (WUE) was improved under elevated CO_2 in leaves from all species. Elevated CO_2 + temperature also promoted enhanced WUE in *U. decumbens* and *M. maximus* leaves, but to a less extended magnitude (Table 2).

3.3. Non-structural carbohydrates content

The main soluble carbohydrates detected in leaves of all species were sucrose, glucose and fructose (Fig. 4A, B and C). Soluble sugar content did not change for *U. brizantha* and *U. decumbens* under different environmental conditions. However, *M. maximus* plants grown under elevated temperature showed a decrease of 53.6% in sucrose (Fig. 4A), 50.6% in glucose (Fig. 4B) and 49.8% in fructose content (Fig. 4C). Also, *M. maximus* plants exposed to elevated CO_2 + temperature showed an increase of 53.5% in leaf sucrose content related to the plant control and elevated CO_2, and 157.9% related to elevated temperature (Fig. 4A). Starch content in leaves of all species did not change with different environmental conditions (Fig. 4D).

3.4. Cellulose and lignin contents

Leaf cellulose content was unchanged in all species under different environmental conditions (Fig. 5A). For lignin content, *U. brizantha* plants showed a decrease when subjected to all different environmental conditions. *U. decumbens* and *M. maximus* showed no changes in leaf lignin content for any environmental condition (Fig. 5B).

3.5. Principal component analysis

Principal component analysis (PCA) of the entire dataset showed that the first and second principal components (PCs) explained 36.87% and 19.82% of the total variance, respectively (Fig. 6A) and revealed that differences were more evident between species, corroborating the fact that these species have intrinsic differences in their resources use.

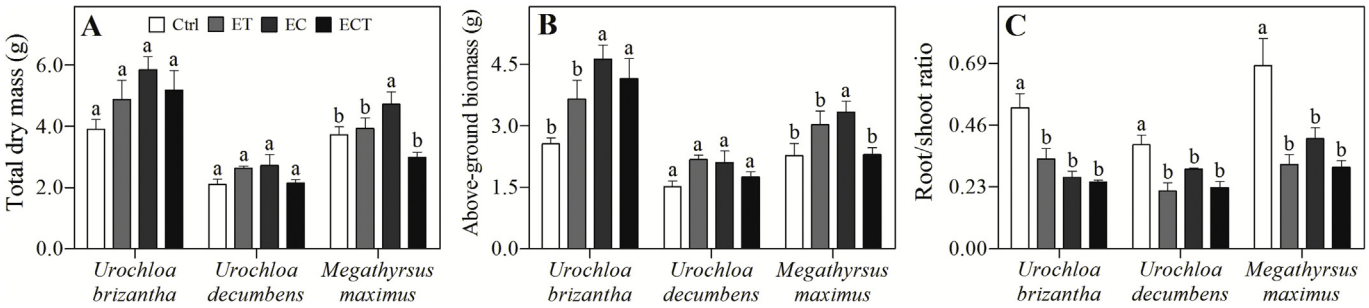

Fig. 2. Total dry mass (A), above-ground biomass (B) and root/shoot ratio (C) of three invasive grass species grown under elevated CO_2 concentration and/or elevated temperature. Results represent mean ± SEM (n = 4). Different letters indicate statistical differences ($P < 0.05$) between environmental conditions (Ctrl: Control; ET: Elevated temperature; EC: Elevated CO_2; ECT: Elevated CO_2 + temperature).

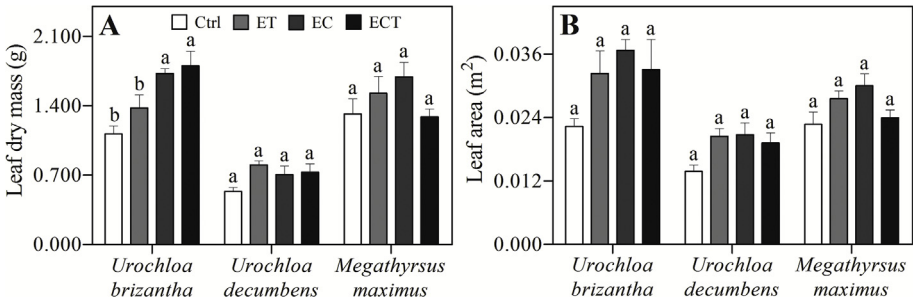

Fig. 3. Leaf dry mass (A) and Leaf area (B) of three invasive grass species grown under elevated CO_2 concentration and/or elevated temperature. Results represent mean ± SEM (n = 4). Different letters indicate statistical differences ($P < 0.05$) between environmental conditions (Ctrl: Control; ET: Elevated temperature; EC: Elevated CO_2; ECT: Elevated CO_2 + temperature).

Table 2
Gas exchange parameters [net photosynthetic assimilation (*A*), stomatal conductance (g_s), rate of transpiration (*E*) and water use efficiency (WUE)] of three invasive grasses species grown under elevated CO_2 concentration and/or elevated temperature treatments.

	A (μmol CO_2 m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	*E* (mmol H_2O m^{-2} s^{-1})	WUE (mmol CO_2 mol H_2O^{-1})
Urochloa brizantha				
Ctrl	10.52 ± 0.51 **c**	0.0104 ± 0.0006 **b**	0.4188 ± 0.0242 **b**	25.23 ± 0.53 **b**
ET	12.24 ± 0.36 **c**	0.0123 ± 0.0005 **a**	0.5210 ± 0.0275 **b**	23.71 ± 0.83 **b**
EC	13.86 ± 0.89 **b**	0.0099 ± 0.0006 **b**	0.4563 ± 0.0324 **b**	30.74 ± 1.52 **a**
ECT	18.43 ± 0.45 **a**	0.0140 ± 0.0004 **a**	0.6957 ± 0.0152 **a**	26.53 ± 0.63 **b**
Urochloa decumbens				
Ctrl	14.22 ± 0.87	0.0246 ± 0.0019 **b**	0.8732 ± 0.0688 **b**	16.49 ± 0.63 **c**
ET	17.11 ± 2.01	0.0332 ± 0.0038 **a**	1.2398 ± 0.1426 **a**	13.84 ± 0.54 **c**
EC	14.52 ± 1.37	0.0158 ± 0.0017 **b**	0.6567 ± 0.0639 **b**	22.24 ± 0.80 **a**
ECT	17.03 ± 1.15	0.0214 ± 0.0020 **b**	0.9282 ± 0.0801 **b**	18.81 ± 1.40 **b**
Megathyrsus maximus				
Ctrl	9.36 ± 0.75	0.0166 ± 0.0011 **b**	0.5965 ± 0.0376 **b**	15.61 ± 0.55 **b**
ET	9.99 ± 0.69	0.0213 ± 0.0016 **a**	0.8115 ± 0.0624 **a**	12.41 ± 0.50 **c**
EC	11.79 ± 0.62	0.0139 ± 0.0007 **b**	0.5634 ± 0.0314 **b**	20.97 ± 0.31 **a**
ECT	10.63 ± 1.34	0.0145 ± 0.0017 **b**	0.6374 ± 0.0765 **b**	16.59 ± 0.88 **b**

Ctrl: Control; ET: Elevated temperature; EC: Elevated CO_2; ECT: Elevated CO_2 + temperature. Results represent mean ± SEM (n = 6). Absence of letters indicate no statistical differences; different letters indicate statistical differences ($P < 0.05$) between environmental treatments.

M. maximus was more correlated to the PC2 and *U. brizantha* to PC1. *U. decumbens* was out of the two PCs. Also, *M. maximus* was more correlated to the variables fructose content, root/shoot ratio, cellulose content, culm diameter, glucose content, leaf area and starch content, *U. brizantha* was more correlated to the variables total dry mass, leaf dry mass, sucrose content, lignin content, WUE, total height, number of leaves and *A*, and *U. decumbens* was more correlated to the variables *A*, the number of tillers, *E* and g_s (Fig. 6A).

Because the first PCA of the entire dataset separated species, new PCAs were performed, one for each species separately. For *U. brizantha*, the PCA separated the control treatment of the environmental treatments. Control treatment was more related to the PC2. According to this PCA, all environmental treatments seem to have the same effects on variables, differing from control treatment (Fig. 6B). For *U. brizantha*, the variables lignin, cellulose and starch content were positively correlated to the control treatment. The variables g_s, *E*, *A*, fructose and glucose content and leaf dry mass were positively correlated to each other and to ECT treatment, and the variables, total height, sucrose

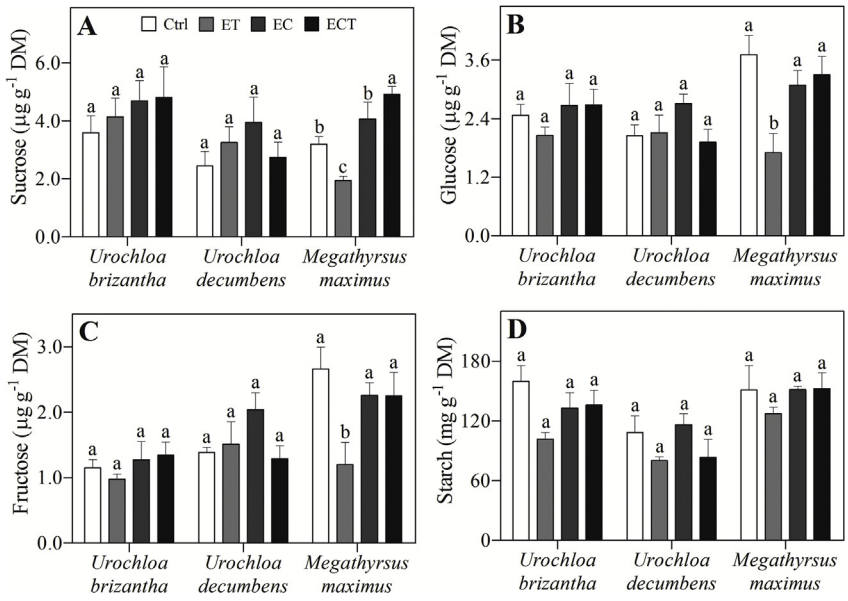

Fig. 4. Non-structural carbohydrates leaf content (A – sucrose, B – glucose, C – fructose and D – starch) of three invasive grass species grown under elevated CO_2 concentration and/or elevated temperature. Results represent mean ± SEM (n = 4). Different letters indicate statistical differences ($P < 0.05$) between environmental conditions (Ctrl: Control; ET: Elevated temperature; EC: Elevated CO_2; ECT: Elevated CO_2 + temperature).

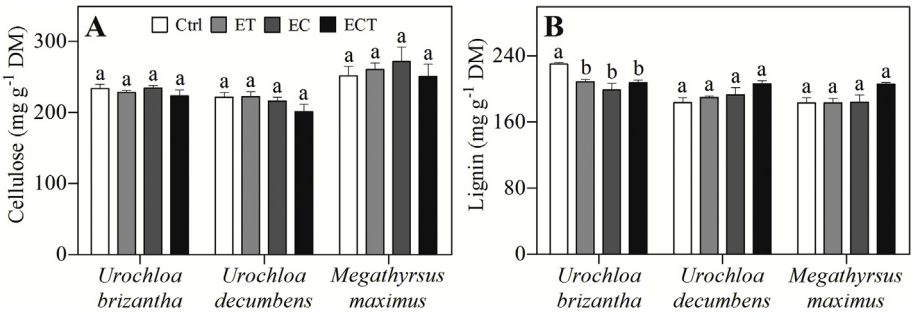

Fig. 5. Cellulose (A) and lignin (B) leaf content of three invasive grass species grown under elevated CO_2 concentration and/or elevated temperature. Results represent mean ± SEM (n = 4). Different letters indicate statistical differences ($P < 0.05$) between environmental conditions (Ctrl: Control; ET: Elevated temperature; EC: Elevated CO_2; ECT: Elevated CO_2 + temperature).

content, leaf area and WUE were positively correlated to each other and to EC and ET treatments. The variable leaf dry mass was negatively correlated to the variables root/shoot ratio, lignin and cellulose content, and the variables g_s, *E*, *A*, fructose and glucose content were negatively correlated to the variable starch content. The variables g_s, *E*, *A*, WUE, fructose, glucose and sucrose content, leaf dry mass, total height and leaf area were negatively correlated to the control treatment (Fig. 6B).

For *U. decumbens*, PCA did not separate any treatment. The variables glucose and fructose content, g_s and *E* were closely related. The variables glucose and fructose content, leaf area, total dry mass and leaf dry mass were positively correlated to each other and negatively correlated to variable *A*; the variables sucrose and lignin content were negatively correlated to each other; the variables g_s, *E* and cellulose content were positively related to each other and negatively correlated to the variables total height, WUE, starch content and root/shoot ratio, that were

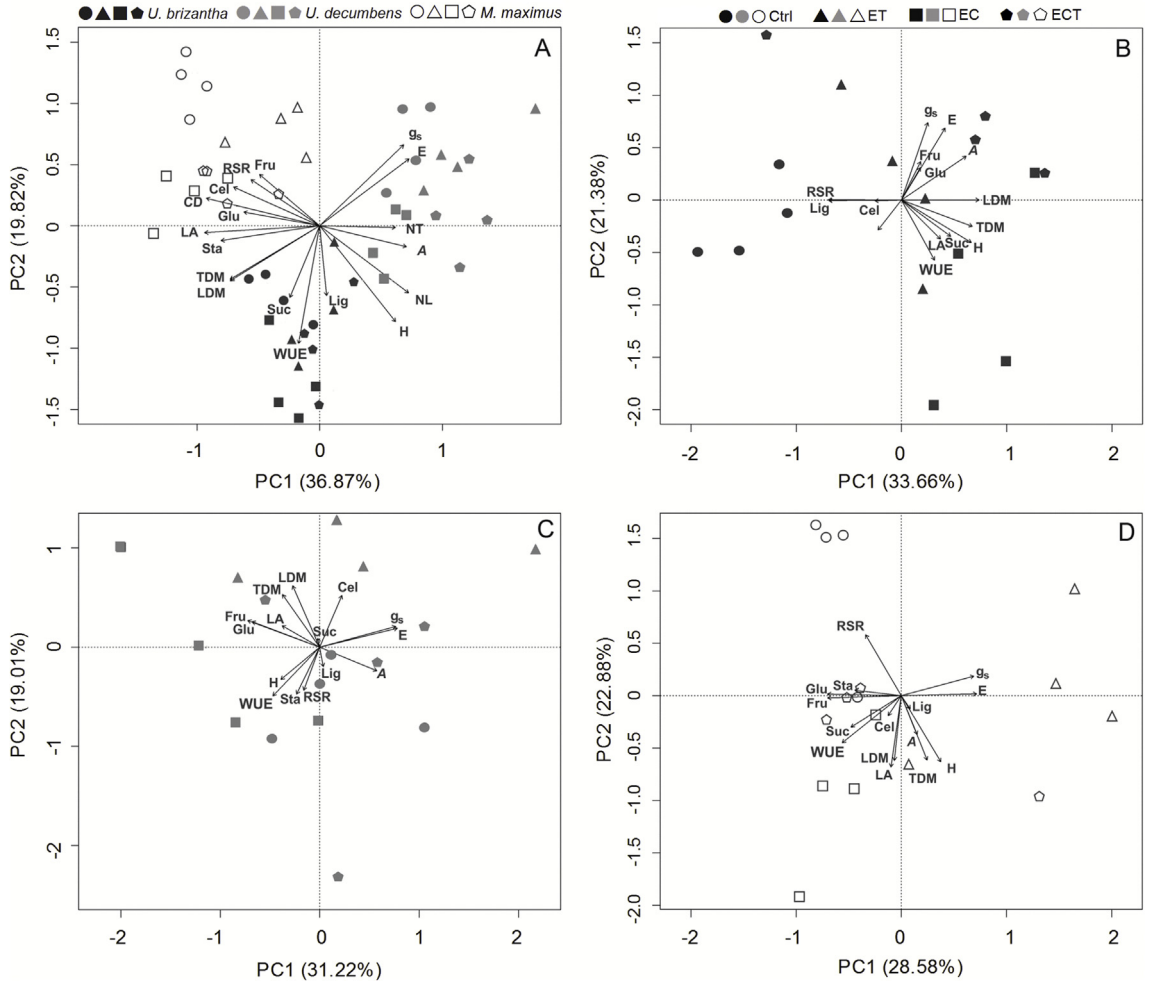
U. brizantha U. decumbens M. maximus
A
PC2 (19.82%)
PC1 (36.87%)
gs E Fru RSR Cel CD Glu LA Sta TDM LDM Suc Lig NT A NL H WUE
Ctrl ET EC ECT
B
PC2 (21.38%)
PC1 (33.66%)
gs E A Fru Glu RSR Lig Cel LDM TDM LA Suc H WUE
C
PC2 (19.01%)
PC1 (31.22%)
LDM TDM Cel Fru Glu LA Suc gs E H Lig A WUE Sta RSR
D
PC2 (22.88%)
PC1 (28.58%)
RSR gs E Glu Sta Fru Lig Suc Cel WUE A LDM LA H TDM

Fig. 6. Principal component analysis on plant functional traits of three invasive grass species grown under elevated CO_2 concentration and/or elevated temperature (Circles: control – Ctrl; triangles: elevated temperature – ET; squares: elevated CO_2 – EC; pentagons: elevated CO_2 + temperature – ECT). The first two principal components (PC1 and PC2) are shown. **A** – PCA for all species and treatments (Black symbols: *Urochloa brizantha*; gray symbols: *Urochloa decumbens*; white symbols: *Megathyrsus maximus*). **B** – PCA for *Urochloa brizantha*; **C** – PCA for *Urochloa decumbens*; **D** – PCA for *Megathyrsus maximus*. Variables analyzed: *A* – net carbon assimilation; Cel – cellulose content; CD – culm diameter; E – rate of transpiration; Fru – fructose content; g_s – stomatal conductance; Glu – glucose content; H – total plant height; LA – leaf area; LDM – leaf dry mass; Lig – lignin content; NL – number of leaves; NT – number of tillers; RSR – root/shoot ratio; Suc – sucrose content; Sta – starch content; TDM – total dry mass; WUE – water use efficiency. For PCA shown in B, C and D, variables CD, NL and NT were omitted because they were not influenced by treatments for any species. Symbols with the same type and the same color represent the number of replicates for each variable (n = 4).

positively correlated to each other (Fig. 6C).

For *M. maximus*, PCA separated the control treatments in the 1st quadrant; ET treatment in the 2nd quadrant; and EC treatment in the 4th quadrant. The variables glucose, fructose and starch content; leaf dry mass and leaf area; *A* and total dry mass; lignin content and total height were closely related, respectively. The variables cellulose and sucrose content, WUE, leaf dry mass and leaf area were positively correlated; the variables g_s and *E* were positively correlated; the variables total dry mass, *A*, lignin content and total height were positively correlated and negatively correlated to the variable root/shoot ratio; and the variables starch, glucose and fructose content were negatively correlated to the variable *E* (Fig. 6D).

4. Discussion

Temperature and CO_2 changes predicted for 2100 and simulated in OTCs induced an increase in height for *U. brizantha* and *M. maximus*. Plant height is an important parameter since it is related to the greater light absorption and shading of the competitors (Westoby et al., 2002). Similar results were found for cogongrass, another invasive C4 grass under elevated CO_2 (Runion et al., 2016). Elevated CO_2 also induced an increase in total biomass and above-ground biomass of *M. maximus*, although this higher growth was not reflected in greater leaf dry mass neither in greater leaf area. Moreover, *U. brizantha* plants subjected to elevated CO_2 treatments (EC and ECT) presented higher leaf dry mass and above-ground biomass without, however, resulting in higher total biomass. Similar results were presented by common reed plants (*Phragmites australis*) (Eller et al., 2012). Higher growth under elevated CO_2 is commonly observed in C3 species (Leakey et al., 2009; Schädel et al., 2010; Grombone-Guaratini et al., 2013; Kimbal, 2016), which have limited photosynthesis by photorespiration in current concentrations of atmospheric CO_2. However, some studies have also demonstrated that C4 species are also able to respond positively to increased CO_2 concentrations (Souza et al., 2013; Runion et al., 2016). Results of large-scale experiments, however, show large variations and clearly demonstrated that the increase in CO_2 did not necessarily promote plant growth (Leakey et al., 2009; Kimbal, 2016).

Urochloa brizantha was the only species to show improvement in carbon assimilation (*A*) under elevated CO_2, and increased CO_2 and temperature jointly (ECT treatment) resulted in even more intense responses. Improved water use efficiency (WUE) under elevated CO_2 was measured in all studied species. One potential benefit of growth under elevated CO_2 is the reduced stomatal conductance (g_s), decreasing transpiration and improving WUE (Leakey et al., 2009). Although a significant reduction in g_s or *E* had not been observed, there was a trend for such responses under elevated CO_2 which probably resulted in better WUE for all species. Contrastingly, since effects in *E* were not observed for *U. brizantha*, the enhancement in WUE was probably a reflection of the enhancement in *A* presented by these species under elevated CO_2 treatments, as shown by positive correlation between these variables on PCA (Fig. 6B).

Elevated temperature treatment (ET) induced an increase in g_s and *E* of *U. decumbens* and *M. maximus*, but these increases did not result in reduced WUE neither in enhanced *A*. Similar results were observed for orange plants (Ribeiro et al., 2012). It might be expected that warm temperatures cause increases in stomatal conductance due to increased evaporative demands and a consequent imbalance of water relations in leaves (Jones, 1998). This lack of reduction in WUE and carbon assimilation under elevated temperature indicates that the increase in g_s and *E* did not harm plant water status and that the increase in 3 °C did not configure thermal stress. Also, at higher temperatures, plants show higher stomatal conductance as a way to dissipate excess heat on leaf surfaces (Morison and Lawlor, 1999). As plants were well hydrated, increased transpiration did not represent any imbalance in water relations, similarly to the results observed for orange by Ribeiro et al. (2012). Contrastingly, conditions of well hydrated soil are not the case in Brazilian Cerrado, where there is a well-defined dry season. Under these conditions, the greater WUE can benefit these species in competition with native ones.

The main soluble sugars identified in all species, glucose, fructose and sucrose, coincided with the profile obtained for tropical grasses by Moraes et al. (2012). These authors found that the fraction of neutral soluble carbohydrates analyzed for twenty-four grass species was composed of glucose, fructose, sucrose, maltose, raffinose and a series of linear oligosaccharides which co-eluted with the maltose-based compounds. Raffinose and stachyose were also found in this study, but in very low amounts (data not shown). In general, species that showed a positive growth and/or positive photosynthetic responses to elevated CO_2 had non-structural carbohydrate (NSC) content significantly higher in leaves (Oliveira et al., 2010; Grombone-Guaratini et al., 2013). NSC are the primary energy available for growth and dry matter yield in grasses and are also associated with tolerance to environmental stresses (Moraes et al., 2012). However, elevated CO_2 did not increase the amount of NSC, even in *M. maximus,* which had enhanced growth. This species only showed an increased sucrose content at elevated CO_2 + temperature and the different environmental conditions did not induce differences in NSC content for *U. brizantha* and *U. decumbens*.

Elevated temperature reduced sucrose, glucose and fructose contents in *M. maximus* leaves as it was shown by the negative correlation between these variables and ET treatment on PCA (Fig. 6D). NSC content and composition are influenced by several factors, including genetic traits, photosynthetic efficiency, source-sink partitioning, as well as various abiotic factors. When moisture is not limited, temperature is one of the most crucial environmental factors (Moraes et al., 2012). High temperature modifies carbon metabolism enzymes, starch accumulation, and sucrose synthesis through specific genes that down-regulate the carbohydrates metabolism (Ruan et al., 2010). Reductions in NSC content were also observed for tomato plants (Jie et al., 2012) at a 10 °C temperature rise. In both studies, the authors attribute such response to a decrease in photosynthesis by the inhibition of RuBisCO genes expression and to an increase in sucrose phosphate synthase activity, which inhibited fructose and glucose formation and induced a slight increase in sucrose levels in the leaves of stressed plants (Jie et al., 2012). However, there is no change evidence in photosynthetic carbon assimilation (*A*) or reduction in total biomass or leaf biomass in *M. maximus*, grown at elevated temperature that could be related to such stress responses.

Depending on the source-sink balance, NSC can be accumulated in leaves or easily mobilized to other plant parts (Moraes et al., 2012). Sucrose is the most important form of carbon translocated to developing and/or storage in organs (Fallahi et al., 2008). The higher sucrose content in *M. maximus* exposed to a 3 °C temperature increase (ECT treatment) could be understood as a consequence of the large carbon requirements of growing organs (stems) acting as sinks. Stem is a major sink for photoassimilates during the vegetative and early reproductive phases (Wang et al., 2016). Although stem carbohydrate content has not been quantified, there was significant increase in such organ biomass (see supplementary material), which corroborates our hypothesis. In addition, the decreased sucrose, glucose and fructose contents in this species at elevated temperature may also indicate carbohydrates mobilization through phloem to meet the carbon demand of sink organs.

Environmental treatments did not induce changes in the starch content. All the species accumulated more starch than reducing sugars in leaves, as shown by the amounts recorded by plants grown under control treatment. This seems to be an intrinsic trait common in tropical grasses, especially species of Paniceae tribe (Moraes et al., 2012), to which all the species studied belong. The composition of carbohydrate reserve in grasses is also influenced equally by genetic and environmental factors. While sucrose and fructans are reserve constituents predominant in grasses of temperate climate zones, sucrose and starch are predominantly accumulated in grasses originated from tropical zones (Moraes et al., 2012).

Cellulose content was not affected by any of the environmental treatments. Unlike NSC, the overall response of total structural carbohydrates (SC) content to changes in carbon supply seems to be weaker. Schädel et al. (2010) found constant concentrations of hemicellulose with increasing CO_2 in two of four grass species studied. Poorter et al. (1997) found no alterations in SC concentrations under high CO_2 in leaves of herbaceous crops and wild species, as well as in leaves of woody species. Naudts et al. (2013) found no differences in SC levels between the current and future climates in the grasslands communities studied. However, Poorter et al. (1997) and Naudts et al. (2013) do not differentiate between hemicellulose and cellulose within the polysaccharides of the cell wall fractions analyzed. The insoluble sugar concentration reflects the levels of structural carbohydrates and starch, and is an indicator of sugar storage and cell wall formation activities (Naudts et al., 2013).

Regarding lignin content, only *U. brizantha* showed reduction in this parameter under all treatments. Studies addressing lignin content under elevated CO_2, report various types of responses. Elevated CO_2 increased the lignin content in young poplar (Richet et al., 2012) and decreased lignin content in beech (Blaschke et al., 2002). Poorter et al. (1997) did not observe changes in lignin content for any of the 27 species studied. At the physiological level, the lignification process marks an important step in plant development: tissue maturation (Blaschke et al., 2002). For beech, the observed reduction in leaf lignin content suggests that elevated CO_2 delayed structural compounds, including lignin, accumulation in leaves and, thus, the juvenile stage was slightly longer (Blaschke et al., 2002). The observed reduction in lignin content in *U. brizantha* can also be related to delayed leaf maturation. Although no significant differences were observed, there was an increasing trend in leaf area for this species in the same treatment for which the lignin content was decreased. As this species also showed higher rates of carbon assimilation (*A*) under elevated CO_2 treatments, it is likely that the assimilated carbon is being incorporated into NSC at the expense of lignin synthesis. Since lignin is a final metabolic product and is therefore not reused, plants may preferably invest the excess carbon in growth and renewable resources such as sugar and starch, particularly in foliar tissues (Blaschke et al., 2002).

5. Conclusion

Taken together, results suggest that despite having C4 metabolism, all three species were responsive to elevated CO_2, with metabolic responses differing according to the species. Temperature increases also promoted changes in the species responses, increasing the height of *U. brizantha* and *M. maximus* and the stomatal conductance of the three species. These responses show that the studied species are tolerant to high temperatures, and some of them could even benefit from temperature increase. *Urochloa decumbens* was the species that least benefited from climate change effects, improving only WUE under doubled CO_2 concentration. However, as this species has already an aggressive invader potential, it is suggested that it will not be strongly affected by climate change, and also possible that it invades areas which are not subjected to severe droughts. Invasive species are more likely to have greater genetic diversity and physiological plasticity therefore they are more likely to be able to adapt to a changing environment. Moreover, the distribution of *U. brizantha* and *M. maximus* may be enlarged/expanded if native species co-occurring with them do not present a similar response. Comparative studies between these species and highly responsive native species to climate change, as well as other invasive species with C3 metabolism that can be more responsive are needed in an attempt to model the future occupation of the Brazilian Cerrado.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.plaphy.2018.04.042.

Contributions

The study was conceived by MGCF and MG. APF and MAM performed the experiments. APF computed and analyzed data. APF, MAN, MG and MGCF wrote the manuscript.

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